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Effects of Cigarette Smoking on Glomerular Structure and Function in Type 2 Diabetic Patients

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Abstract. Prospective studies have established smoking as an independent risk factor for diabetic nephropathy, suggesting an adverse effect of smoking on glomerular structure and function. To test this hypothesis, this study evaluated GFR, metabolic profile, and smoking habits in 96 patients with type 2 diabetes and abnormal albumin excretion rate (AER). All patients underwent percutaneous kidney biopsy: mesangial fractional volume [Vv (mes/glom)] and glomerular basement membrane (GBM) width were estimated by electron microscopic morphometric analysis; interstitial fibrosis was estimated semiquantitatively by light microscopy. Forty-eight patients were smokers. Compared with nonsmokers, smokers had higher values of HbA_{1c} ($P = 0.002$), AER ($P = 0.026$), GFR ($P = 0.004$), and GBM width ($P = 0.002$); moreover, GFR was higher in current smokers than in former smokers ($P = 0.001$), and GBM width was related to heavy smoking ($F = 5.4$; $P = 0.006$). Multiple linear regression analyses revealed that HbA_{1c} was associated with fasting blood glucose (β coef $= 0.52$; $P < 0.001$), smoking habit (β coef $= 0.31$; $P < 0.001$), insulin therapy (β coef $= 0.22$; $P = 0.012$), and male gender (β coef $= -0.20$; $P = 0.020$); AER was related to Vv (mes/glom) (β coef $= 0.32$; $P = 0.003$), GBM width (β coef $= 0.28$; $P = 0.016$), and interaction between smoking habit and HbA_{1c} (β coef $= 0.24$; $P = 0.040$). GFR was negatively correlated with Vv (mes/glom) (β coef $= -0.57$; $P < 0.001$) and age (β coef $= -0.29$; $P = 0.001$) and positively correlated with GBM width (β coef $= 0.27$; $P = 0.012$), heavy current smoking (β coef $= 0.24$; $P = 0.028$), and HbA_{1c} (β coef $= 0.28$; $P = 0.040$); GBM width was explained by Vv (mes/glom) (β coef $= 0.53$; $P < 0.001$), interaction between heavy smoking and HbA_{1c} levels (β coef $= 0.25$; $P = 0.003$), and diabetes duration (β coef $= 0.23$; $P = 0.010$). Smoking habit did not affect the index of interstitial fibrosis. In conclusion, cigarette smoking affects glomerular structure and function in type 2 diabetes and may be an important factor for the onset and progression of diabetic nephropathy.

Several observations suggest that the kidney, in addition to other peripheral vascular beds, is another target organ of smoking. Acute and chronic smoking has been documented to be associated with renal functional impairment, probably mediated by smoking-induced changes in vasoactive hormones (1–3). Cross-sectional and longitudinal studies have shown that smoking has an adverse effect on renal outcome in essential hypertension, primary and secondary nephropathies, and on graft and patient survival in renal transplant recipients (4–9). In diabetic renal disease, smoking emerged as an independent risk factor for the onset of microalbuminuria, the acceleration in the rate of progression from microalbuminuria to proteinuria, and subsequent renal failure both in type 1 and type 2 diabetes (10,11).

The presence of microalbuminuria is the earliest clinical marker of renal injury in diabetic nephropathy; it is therefore conceivable that the documented association between smoking and microalbuminuria in diabetic patients is the consequence of a smoking-induced glomerular damage. To test this hypothesis, we studied by a cross-sectional design the relationships among smoking habit, urinary albumin excretion rate, GFR, and glomerular ultrastructure in white type 2 diabetic patients.

Materials and Methods

Patients

We studied 96 white type 2 diabetic patients (70 men and 26 women; age, 57.8 ± 7.5 yr): 74 with persistent microalbuminuria, and 22 with proteinuria.

Diabetic patients were diagnosed as having type 2 diabetes when the onset was after age 40 yr and when they did not require insulin in the first 2 yr after diagnosis. Insulin-treated patients with normal body weight had a glucagon test performed to confirm the diagnosis of type 2 diabetes (when C peptide levels were normal). Body mass index (BMI) was calculated using the formula: Weight (Kg)/Height^2 (m^2). Patients were defined as microalbuminuric when albumin excretion rate was between 20 and 200 μg/min and as proteinuric when albumin excretion rate was above 200 μg/min in at least two of three sterile 24-h urine collections. Patients were defined hypertensive when BP values were >130/85 mmHg or when antihypertensive therapy was used (angiotensin-converting enzyme [ACE] inhibitors or angiotensin receptors blockers alone or associated with dihydropyridine calcium antagonists and/or diuretics, α- and β-blockers). Patients were admitted to the Department of Medical and Surgical Sciences at the Uni-

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versity of Padova, where medical history, physical examination, and smoking habit questionnaire were performed. Smoking history was derived from yes/no response to the question "Have you ever smoked as much as one cigarette a day for as long as 1 yr?" and "Do you smoke cigarettes now?" Smokers recorded the number of cigarettes smoked each day. Subjects were asked to record the age at which they started to smoke, and those who stopped smoking were asked to record the age at which they quit. Forty-eight subjects (42 men, 6 women) smoking ≥10 cigarettes/d since young adult age were classified as cigarette smokers (1): 22 were current smokers, and 26 were former smokers who had quit smoking for 5 to 7 yr and were not exposed to passive smoking. The smokers were subdivided into 15 moderate smokers (10 to 19 cig/d; 7 current and 8 former smokers) and in 33 heavy smokers (≥20 cig/d; 15 current and 18 former smokers). In the test, we used the smoking habit term to indicate the cigarette smokers independently from cigarette dose (moderate or heavy) and/or current or former status.

During admission, percutaneous kidney biopsy and renal functional studies were performed. Twenty-seven (14 men and 13 women; age, 56 ± 10 yr) white normal subjects served as controls. They were living related kidney donors at the University of Minnesota.

All patients gave their written informed consent before the study. This study protocol was approved by the Ethics Committee of the University of Padova.

Kidney Function Study and Clinical Profile

Albumin excretion rate (AER) was measured by immunoturbidimetric method on three sterile 24-h urine collections (12); GFR was determined between 8 and 9 a.m. after at least 8 h of nonsmoking by plasma clearance of ^{51}Cr-ethylenediaminetetraacetic acid (^{51}Cr-EDTA); ^{51}Cr radioactivity was measured in duplicate 1-ml aliquots of plasma in a gamma counter (Cobra-5002 CPM, Camberra Packard, Milan, Italy) (13). The normal range in a group of 19 age- and gender-mached normal control subjects was 85 to 135 ml/min per 1.73 m^2.

BP was measured at least ten times with the patients in the supine position, and the values provided are the mean of these repeated measurements. HbA_{1c} was measured by high-pressure liquid chromatography (HPLC) (DIAMAT Analyzer, Bio-Rad, Hercules, CA) to assess metabolic control. Total cholesterol and triglycerides were measured enzymatically on a fully automated multichannel analyser (Hitachi 200, Roche, Milan, Italy).

Renal Biopsy Studies

A kidney biopsy was performed if serum creatinine was <180 μmol/L and if there was absence of other obvious renal diseases (*e.g.*, stone disease, presence of single kidney) and secondary causes of hypertension, including known renal artery stenosis. Tissue was immediately processed for light, electron, and immunofluorescence microscopy. Electron microscopic examination was conducted on tissue fixed in 2.5% glutaraldehyde in Millonig buffer and processed as described previously (14,15). At least three glomeruli of each biopsy were examined (nonsmokers, 3.19 ± 0.39; moderate smokers, 3.2 ± 0.41; heavy smokers, 3.15 ± 0.36). Glomeruli were photographed at a magnification of ×3900 to produce photomontages of the entire glomerular profile. The montages were used to estimate mesangial fractional volume [Vv(mes/glom)] by point counting (normal values, 0.19 ± 0.03) (16). Another set of micrographs, obtained at ×12000 by systematically sampling about 20% of the glomerular profile, was used to estimate glomerular basement membrane (GBM) width (normal values, 310 ± 38 nm) by the orthogonal intercept method (15,16). Tissue for light microscopy was fixed in Zenker's and embedded in paraffin; periodic-acid Schiff (PAS)–stained 2-μm-thick slides were used. The index of interstitial fibrosis was determined as a semiquantitative estimate of the space occupied by fibrosis and cellular tissue separating cortical tubules (0 was used as normal, 1.0 as twice normal space, 2.0 as three times normal, etc.) in each ×500 cortical field. Quarter and half grades were assigned where appropriate for each field, and mean values were obtained for each patient (17). This semiquantitative estimate of interstitial expansion is highly correlated with the morphometric measure of interstitial space (interstitial fractional volume) as previously reported (18). None of these patients had light, immunofluorescent, or electron microscopy findings of any definable renal disease other than diabetic nephropathy.

Statistical Analyses

Statistical analyses were performed with the Statistica stat.soft version 5 data analysis system. Data are expressed as mean and ± SD; AER and the index of interstitial expansion, not normally distributed, are expressed as median and range and were logarithmically transformed before analysis. Univariate analysis was performed by *t* test for independent samples, with one-way ANOVA and the Scheffé test for *post hoc* comparisons within groups. χ^2 test was used for frequency analysis.

In addition, the data were examined by multi-way ANOVA and covariance (ANCOVA) to control the influence of confounding variables by a standard factorial crossed design, with multiple fixed factors and covariates. Finally, analysis was completed by a forward stepwise multiple linear regression; standardized β-coefficients, F values, partial r^2, and adjusted overall R^2 values were obtained for each best-fit regression model. The dependent variables were HbA_{1c}, AER, GBM width, GFR (Table 3). The independent variables were fasting plasma glucose (only for HbA_{1c}), gender, age, diabetes duration, BMI, uricemia, total cholesterol, triglycerides, oral hypoglycemic agents, insulin therapy, MAP and ACE inhibitor therapy, smoking habit, moderate and heavy smoking, current and former smoking. In addition, for AER and GFR also Vv (mes/glom) and GBM width were considered as independent variables. For GBM width, Vv (mes/glom) was included.

Moreover, in multi-way ANOVA and in multiple regression, the interaction between smoking status and fasting glycemia >7.8 mmol/L (19) as factor or HbA_{1c} as independent variable was tested. These analyses were performed with HbA_{1c} as a continuos independent variable to assess the main and confounding effects of poor glycemic control on dependent variables. Statistical significance was defined as a $P < 0.05$.

Results

The clinical and laboratory characteristics of nonsmoking and smoking diabetic patients are summarized in Table 1. Compared with nonsmokers, smokers had significantly higher values of HbA_{1c}, AER, GBM width, and GFR and lower serum creatinine values. No significant differences emerged between the two groups for age, diabetes duration, BMI, BP (MAP), cholesterol and triglyceride levels, Vv (mes/glom), antihypertensive treatment, and index of interstitial fibrosis; there was no difference between former and current smokers with regard to the same parameters, except for GFR values, which were significantly higher in current compared with former smokers (109.0 ± 21.7 *versus* 93.2 ± 19.2 ml/min per 1.73 m^2) and nonsmokers ($F = 7.4$; $P = 0.001$) (Figure 1). Interestingly,

Table 1. Clinical and laboratory characteristics of nonsmoking and smoking diabetic patients[a]

	Nonsmokers	Smokers	*t*	*P*
No. of patients	48	48		
Gender (M/F)	28/20	42/6		
Age (yr)	58.8 ± 7.1	57.8 ± 7.1	0.69	0.49
BMI (kg/m^2)	26.7 ± 8.2	27.7 ± 5.9	0.69	0.49
Diabetes duration (yr)	11.6 ± 7.0	9.4 ± 7.0	1.54	0.13
GFR (ml/min per 1.73 m^2)	87.7 ± 22.6	101.0 ± 22.1	2.92	0.004
S-creatinine (μmol/L)	88.4 ± 17.7	79.6 ± 17.7	2.45	0.016
Total Cholesterol (mmol/L)	6.2 ± 1.3	5.7 ± 1.2	1.78	0.078
Triglycerides (mmol/L)	2.5 ± 1.2	2.2 ± 1.1	1.26	0.21
Uricemia (mmol/L)	279.6 ± 77.3	291.5 ± 77.3	0.75	0.45
Fasting glycemia (mmol/L)	10.5 ± 3.4	10.6 ± 3.8	0.13	0.90
HbA_{1c} (%)	7.6 ± 1.5	8.6 ± 1.6	3.16	0.002
MAP (mmHg)	108.5 ± 8.6	106.8 ± 11.1	0.84	0.40
AER (μg/min)	40.0 (22 to 1528)	75.8 (30 to 2107)	2.25[d]	0.026
Vv (mes/glom)	0.27 ± 0.06	0.26 ± 0.06	0.82	0.42
GBM width (nm)	398.0 ± 92.5	461.0 ± 104.4	3.13	0.002
Index of interstitial fibrosis	0.50 (0 to 3.5)	0.40 (0 to 2.8)	0.13[d]	0.90
Treatment for hypertension	86.4%	86.7%		
ACEI or ARB[b]	61.4%	64.4%		
Calcium antagonists[c]	25.0%	22.3%		

[a] Values are given as prevalence in % for antihypertensive treatment, as median (and range) for AER and index of interstitial fibrosis and as mean ± SD for other all variables. BMI, body mass index; MAP, mean arterial pressure; AER, albumin excretion rate, Vv (mes/glom), mesangial fractional volume; GBM, glomerular basement membrane; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers.

[b] Alone or in association with calcium antagonists, diuretics, or α- and β-blockers.

[c] Alone or in association with diuretics.

[d] *t* test performed after logarithmic transformation.

when cigarette smoking patients were divided in two subgroups according to the number of smoked cigarettes, a significant increase in GBM width emerged in heavy smokers, in comparison with moderate smokers and nonsmokers (471.0 ± 113.3 *versus* 438.6 ± 80.9 *versus* 398.0 ± 92.5 nm) ($F = 5.4$; $P = 0.006$) (Figure 2), whereas AER, HbA_{1c}, and GFR were not related to the smoking dose.

The data were examined by multi-way ANOVA and covari-

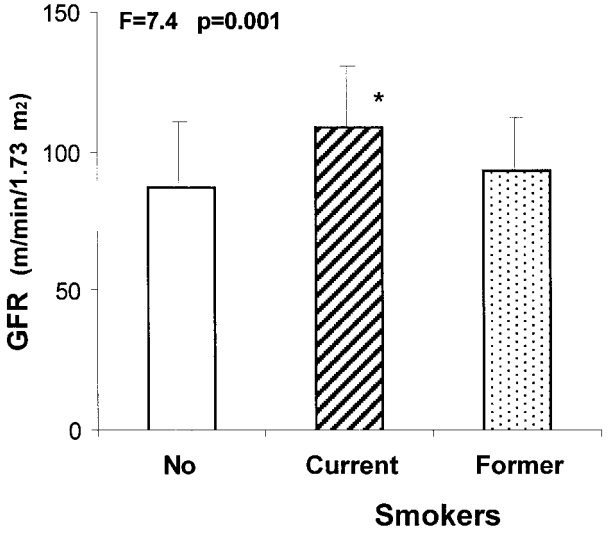

Figure 1. Effect of current smoking on GFR. *$P < 0.01$ *versus* other groups.

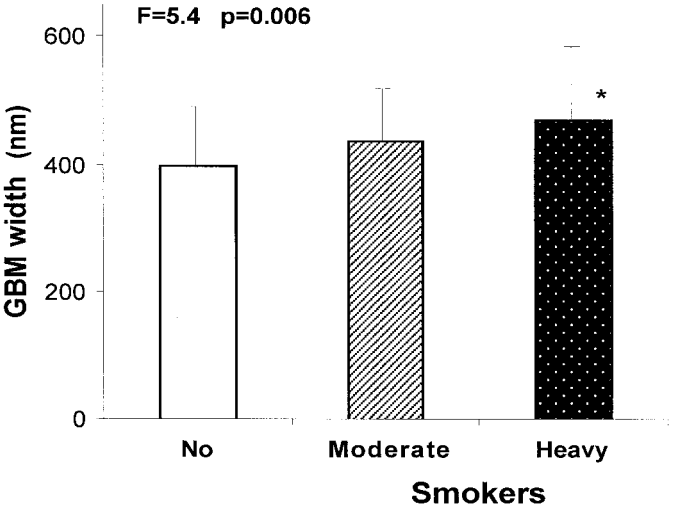

Figure 2. Effect of heavy smoking (≥20 cigarettes/d) and moderate smoking (10 to 19 cigarettes/d) on glomerular basement membrane (GBM) width. *$P = 0.01$ *versus* nonsmokers.

ance to determine whether the parameters that were different between smokers and nonsmokers (HbA_{1c}, AER, GBM width, and GFR) were influenced by smoking besides confounding effects of other clinical and demographic variables (Table 2). HbA_{1c}, after adjustment for gender, age, BMI, diabetes duration, antidiabetic therapy, was affected by smoking ($F = 9.8$; $P = 0.003$) and fasting blood glucose >7.8 mmol/L ($F = 14.1$; $P < 0.001$), whereas the interaction between smoking and glycemia was NS. AER, after adjustment for gender, age, MAP, diabetes duration, ACE inhibitor therapy, and index of interstitial expansion were significantly influenced only by smoking habit ($F = 4.6$; $P = 0.035$) independently of cigarette dose and status (current or former). Adjustment for HbA_{1c} ($F = 3.15$; $P = 0.08$), GBM width ($F = 1.4$; $P = 0.25$), and Vv (mes/glom) ($F = 8.6$; $P = 0.005$) eliminated the significant effect of smoking on AER ($F = 2.07$; $P = 0.15$). Heavy smoking ($F = 4.9$; $P = 0.011$), independently of status (current or former), emerged to have significant effect on GBM width

Table 2. Results by multi-way analysis of variance (ANOVA) and covariance (ANCOVA)[a]

Dependent Variable	Factors	Covariates	F	*P*
$HbAl_c$	Smoking habit		9.79	0.003
	Fasting glycemia >7.8 mmol/L		14.12	<0.001
	Smoking x glycemia >7.8 mmol/L		0.30	0.58
		Gender	5.47	0.022
		Age	1.25	0.27
		BMI	0.50	0.48
		Uricemia	2.93	0.09
		Total cholesterol	0.03	0.86
		Triglycerides	3.87	0.053
		Diabetes duration	0.05	0.82
		Insulin therapy	6.22	0.015
		Oral hypoglycemic agents	0.24	0.63
AER	Smoking habit		4.64	0.035
		Gender	0.18	0.67
		Age	0.05	0.83
		MAP	0.14	0.71
		Diabetes duration	8.39	0.005
		ACEI or ARB therapy	0.13	0.72
		Index of interstitial fibrosis	4.95	0.029
GBM width	Heavy smoking		4.89	0.011
		Gender	1.10	0.30
		Age	2.38	0.13
		$HbAl_c$	6.95	0.010
		AER	6.69	0.012
		Diabetes duration	19.92	<0.001
		ACEI or ARB therapy	0.11	0.74
		Vv (mes/glom)	12.61	0.001
		Index of interstitial fibrosis	2.63	0.10
GFR	Current smoking		3.68	0.031
		Gender	0.74	0.39
		Age	19.43	<0.001
		MAP	2.84	0.097
		$HbAl_c$	1.70	0.20
		Diabetes duration	3.65	0.061
		AER	2.22	0.14
		ACEI or ARB therapy	0.16	0.70
		Vv (mes/glom)	14.61	<0.001
		GBM width	6.60	0.013
		Index of interstitial fibrosis	2.65	0.11

[a] Performed to determine whether the parameters that were different between smokers and nonsmokers (HbA_{1c}, AER, GBM width, and GFR) were influenced primarily by smoking besides by confounding effects of other clinical and demographic variables. x, interaction between factors.

after adjustment for diabetes duration, HbA_{1c}, gender, age, Vv (mes/glom), AER, index of interstitial expansion, and antihypertensive treatment. Current smoking ($F = 3.7$; $P = 0.031$) resulted to have a significant effect on GFR after adjustment for gender, age, diabetes duration, MAP, $HbA_{1c,}$ Vv (mes/glom), GBM width, AER, index of interstitial expansion, and antihypertensive treatment.

Multiple stepwise linear regression analysis was carried out to compare the load and sign of relative contribution of each independent variable on HbA_{1c}, AER, GBM width, and GFR (Table 3). HbA_{1c} was positively associated ($F = 20.5$; $P < 0.001$) with fasting plasma glucose, smoking habit, and insulin therapy and negatively associated with male gender. AER was positively related ($F = 15.2$; $P < 0.001$) to Vv (mes/glom), GBM width, and interaction between smoking and HbA_{1c}. Multivariate regression analysis showed that only Vv (mes/glom), diabetes duration, interaction between heavy smoking, and HbA_{1c} were significant independent determinants of GBM width ($F = 23.5$; $P < 0.001$). Finally, GFR was negatively correlated ($F = 17.1$; $P < 0.001$) with Vv (mes/glom) and age and positively correlated with HbA_{1c}, GBM width, and heavy current smoking.

Discussion

This cross-sectional study demonstrates that chronic cigarette smoking in patients with type 2 diabetes is associated with alterations of glomerular structure and function. Compared with nonsmokers, smokers had higher urinary albumin excretion rate and GFR and the thicker GBM, whereas no significant difference in the interstitial lesions emerged between the two groups. The finding of the association between smoking and AER is not surprising. Several previous observations reported a role of cigarette smoking in promoting development and progression of diabetic kidney disease, despite ACE inhibitor therapy (11). This relationship has predominantly been documented in type 1 diabetic patients (10); however, current smoking has also been shown to be a risk factor for the onset of microalbuminuria in type 2 diabetes, independent of age, gender, HBA_{1c}, and diabetes duration (20–22).

More intriguing is the relationship that emerged from our study of cigarette smoking and GFR. Current smoking patients had higher GFR than patients who had stopped smoking and nonsmokers (Figure 1); multivariate analysis showed that GFR, besides being related to GBM width and HbA_{1c}, was also positively related to heavy current smoking. Thus, at this early stage of diabetic nephropathy, smoking appears to counterbalance the effects of age and diabetes on loss in GFR, resulting in what may appear to be a beneficial effect of smoking on GFR. However, this increase in GFR could reflect an increase in intraglomerular capillary pressure or flow, which could accelerate the progression of diabetic nephropathy (23). Reports on the acute effect of smoking on GFR are not uniform. After smoking two cigarettes, GFR and renal plasma flow fell in healthy volunteers, but not in patients with IgA glomerulonephritis (2). Smoking three cigarettes per hour during a 5.5-h period induced no variation of GFR in normotensive type 1 diabetic patients who had been smoking for several years (24). Similarly, the chronic effects of smoking on GFR are controversial. A cross-sectional study of nondiabetic subjects showed that, compared with nonsmokers, cigarette smokers had a significant reduction of renal plasma flow but normal GFR (1). Ekberg *et al.* (25) reported that glomerular hyperfiltration was directly related to smoking and that GFR was directly dependent on the smoking dose in type 1 diabetic patients. Moreover, it has been demonstrated that, in the general population, creatinine clearance in males was higher in current smokers than in former smokers and nonsmokers and that this difference was correlated with the number of cigarettes smoked daily (26). More recently, Bangstad *et al.* (27) reported that young smoking type I diabetic patients had a higher baseline GFR than nonsmokers and that smoking resulted in significant independent risk factors for the subsequent decline in GFR. Other studies have proposed that chronic smoking in diabetes mellitus is associated with the development and progression of diabetic kidney disease and that cessation of smoking reduced the rate of loss of GFR (10,11).

The mechanisms underlying the effects of smoking on GFR and albuminuria are unclear (28,29). Smoking induces the release of vasoconstrictor sympathetic neurotransmitters, causes morphologic and functional changes in blood vessels, such as the induction of proliferation of intimal smooth-muscle cells, decrease in endothelial prostacyclin synthesis, and endothelial derived vascular tone regulators, thus inducing an imbalance between vasodilator and vasoconstrictor vasoactive mediators. Interference with the vascular response to acetylcholine, nitric oxide, and endothelin-1, which is found increased in smoking healthy subjects, has been reported (1). Interestingly, smoking, by the induction of hypoxic stress, may interfere with vascular endothelial growth factor (VEGF) synthesis and activity; VEGF is a potent mitogen for endothelial cells, plays a central role in the regulation of vasculogenesis and vascular permeability, and seems to be involved in diabetic complications (30).

On the basis of these vasoactive effects of smoking, it has been hypothesized that repeated episodes of acute renal hypoperfusion induced by smoking may favor structural alterations of preglomerular vessels and glomerular obsolescence, thus leading to hypertrophy and hyperfiltration of remnant glomeruli (26,29), which would explain the elevated GFR observed in current smokers compared with patients who had stopped smoking and nonsmokers. This hypothesis is supported by autopsy studies (31,32) demonstrating morphopathologic changes in the renal microvasculature in cigarette smokers. In addition, glomerular hyperfiltration has been demonstrated to be a predictor of GBM thickening in adolescents with type 1 diabetes (33). Our data are in keeping with these findings; indeed, the smokers had a smoking dose-dependent increase in GBM width (Figure 2). Multivariate analysis revealed that, besides diabetes duration and Vv (mes/glom), the interaction between heavy smoking and HbA_{1c} levels were significant determinants of GBM thickness, indicating that the presence of both factors together (heavy smoking and HbA_{1c}) had a more than additive effect on GBM width.

GBM thickening, along with mesangial expansion and arte-

Table 3. Multivariate regression analysis of the relationship between HbA_{1c}, AER, GBM width, GFR, and other clinical and demographic variables[a]

Variables dependent	Independent	Statistics Beta	*P*	r^2	R^2
HbA_{1c}	Fasting glycemia	0.52	<0.001	0.31	0.47
	Smoking habit	0.31	<0.001	0.15	
	Insulin therapy	0.22	0.012	0.07	
	Male gender	−0.20	0.020	0.06	
AER	Vv (mes/glom)	0.32	0.003	0.13	0.30
	GBM width	0.28	0.016	0.11	
	Smoking habit x HbA_{1c}	0.24	0.040	0.08	
GBM width	Vv (mes/glom)	0.53	<0.001	0.29	0.42
	Heavy smoking x HbA_{1c}	0.25	0.003	0.10	
	Diabetes duration	0.23	0.010	0.07	
GFR	Vv (mes/glom)	−0.57	<0.001	0.25	0.35
	Age	−0.29	0.001	0.14	
	GBM width	0.27	0.012	0.12	
	Heavy current smoking	0.24	0.028	0.11	
	HbA_{1c}	0.28	0.040	0.06	

[a] Results of a stepwise multiple linear regression by excluding the less significant variables (best fit model). Statistics Beta, Standardized β-coefficient; r^2, partial r-square values (variance explained by each independent variables); Adjusted R^2, overall r-square values (variance explained by all independent variables); x, interaction between independent variables.

riolar hyalinosis, are the structural hallmarks of diabetic nephropathy (34). Moreover, a biochemical derangement in GBM composition leading to abnormal charge permselectivity may represent an important mechanism of abnormal AER. Proteinuria in diabetic patients is associated with a reduction in glomerular charge density, perhaps consequent to nonenzymatic glycosylation of the various GBM proteins, or to a glycosaminoglycan (GAG) metabolism disorder, leading to reduced heparan sulfate content or sulfatation pattern (34,35–37). Interestingly, there is consistent evidence demonstrating that smoking, by hypoxic stress induction, affects GAG metabolism (38,39), perhaps aggravating the structural and biochemical modifications in GBM induced by the diabetic melieu.

Several studies suggest that smoking may influence albuminuria and abnormal renal function through advanced glycation end products, which have been shown to enhance vascular permeability (40,41); recently Scott *et al.* (19) demonstrated that the effect of smoking was enhanced in individuals with poor glycemic control. Our data are in agreement with this hypothesis; indeed, the significant effect of smoking on AER was eliminated after adjustment for HbA_{1c} and morphologic parameters; AER was best explained, in addition to Vv (mes/glom) and GBM width, by interaction between smoking habit and HbA_{1c} with a multiplicative effect of the two variables; moreover, GBM width resulted to be a strong determinant of AER and was significantly associated to the interaction between heavy smoking and HbA_{1c} levels.

In conclusion, this study documents for the first time an association between smoking status and glomerular lesions in patients with type 2 diabetes, linking smoking to GBM thickening. This effect on glomerular structure along with other hemodynamic and nonhemodynamic effects of smoking may contribute to albuminuria and GFR loss. Long-term controlled prospective studies are required to determine whether these effects of smoking on glomerular structure and renal function are predictive of later renal structural and functional outcomes. However, these findings provide new insights on structural renal injury induced by cigarette smoking and should be relevant in clinical practice, considering that smoking is a modifiable risk factor for diabetic renal disease.

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